PATENT COOPERATION TREATY

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From the INTERNATIONAL SEARCHING AUTHORITY

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2004/001765	26.04.2004	26.06.2003

International Patent Classification (IPC) or both national classification and IPC
G01N33/68, C12Q1/44

Applicant
BABRAHAM INSTITUTE

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2004/001765

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was field, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-54 (industrial applicability), 55-59 (partly)

because:

☒ the said international application, or the said claims Nos. 1-54 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 55-59 are so unclear that no meaningful opinion could be formed *(specify)*:

see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1,3-5,14,18,22,29,30,41,42,50-54
	No:	Claims	2,6-13,15-17,19-21,23-28,31-40,43-49,55-59
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-59
Industrial applicability (IA)	Yes:	Claims	55-59
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item III

1. Claims 55-59 do not define the subject-matter clearly and unambiguously (Art.6 PCT) as the compound is not defined by technical features (e.g. structural features) which would clearly characterize the said compound, but rather with the process to identify the said compound. Said definition might well encompass known compounds and does not allow to discriminate between novel compounds and compounds which have been disclosed in the prior art. Furthermore, the application discloses clearly only the peptides corresponding to the sequences listed on pages 48 and 49 of the description. For these reasons, the search has been restricted to peptides comprising the above-mentioned sequences, and the examination will also be restricted thereto.

2. Claims 41 and 42 are related to *in-vivo* methods, therefore an opinion on industrial applicability will not be provided (Rule 67.1(iv) PCT). As these claims are dependent on the main method-related independent claims 1-6, an opinion on industrial applicability will not be provided for claims 1-54.

Re Item V

1. The following documents are referred to in this communication:

   D1 : CHIU VI K ET AL: "Ras signalling on the endoplasmic reticulum and the Golgi" NATURE CELL BIOLOGY, vol. 4, no. 5, May 2002, pages 343-350.

   D2 : KIM STELLA H ET AL: "E-cadherin-mediated cell-cell attachment activates Cdc42" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, no. 47, 24 November 2000, pages 36999-37005.

   D3 : SRINIVASAN SUPRIYA ET AL: "Rac and Cdc42 play distinct roles in regulating PI(3,4,5) P3 and polarity during neutrophil chemotaxis." JOURNAL OF CELL BIOLOGY, vol. 160, no. 3, 3 February 2003, pages 375-385.

   D4: LOCKYER PETER J ET AL: "CAPRI regulates Ca2+-dependent inactivation of the Ras-MAPK pathway" CURRENT BIOLOGY, vol. 11, no. 12, 26 June 2001, pages 981-986.

2. The subject-matter of independent claims 2 and 6 is not novel (Art.33(2) PCT).

D1 (page 343, right-hand column, para.2-page 346, left column, para.1) discloses a method for determining activation of oncogenic H-Ras induced by EGF or insulin, comprising co-expressing H-Ras with the Ras-binding domain (RBD) of c-Raf-1 amino-terminally tagged with green fluorescent protein (GFP) (construct GFP-RBD, fluorescent reporter), and detecting EGF- or insulin-induced redistribution of GFP-RBD from the cytosol and nucleoplasm to the plasma membrane, where the protein H-Ras is located. In one embodiment of the method, the entire secretory apparatus of COS-1 cells was marked with yellow fluorescent protein (YFP), and EGF-induced free resonance energy transfer (FRET) between YFP and GFP was detected.

D2 (page 37001, left column, para.2; figure 6; page 37004, left column, para.2-right-hand column, para.1) discloses a method for determining E-cadherin-induced Cdc42 activation, comprising transfecting cells with the GTPase binding domain of the Wiskott-Aldrich syndrome protein (WASP-GBD) tagged with GFP (WASP-GBD-GFP construct, fluorescent reporter), and detecting E-cadherin-induced fluorescence accumulation in the plasma membrane.

D3 (page 376, right-hand column, para.3- page 377, right-hand column, para.1; figure 3) discloses a method for determining the fMLP-induced Rac activation, comprising transfecting cells with the p-21-binding domain (PBD) of p21-activated kinase (PAK) tagged with GFP (PAK-PBD-GFP construct, fluorescent reporter), and detecting fMLP-induced localization of PAK-PBD-GFP to the cell periphery.

2.1. The subject-matter of dependent claims 7-13, 15-17, 19-21, 23-28, 31-40 and 43-49 is also not novel, as all the technical features of these claims are disclosed by the documents D1-D3 (see above).

2.2 The subject-matter of dependent claims 14, 18, 22, 29, 30, 41 and 42 is novel, as the technical features of these claims are not disclosed by the documents D1-D3.

2.3. Dependent claims 14, 18, 22, 29, 30, 41 and 42, however, do not appear to contain additional features which meet the requirements of inventive step (Art.33(3) PCT), as all the features of these claims fall within the customary practice of the skilled person or are conventional in the art (see D1-D3).

3. The subject-matter of claims 1, 3-5 and 50-54 is novel (Art.33(2) PCT). The cited prior art does not disclose a method for identifying compounds capable of promoting deactivation, inhibiting activation, or inhibiting GTP loading, of a membrane-bound active small GTPase, including Ras, comprising incubating a live cell expressing the GTPase and having a specific reporter thereof in presence of a test compound, and monitoring a change in the association between reporter and GTPase.

4. The subject-matter of independent claim 1 is however not inventive (Art.33(3) PCT).

The difference between claim 1 and each the documents D1-D3 (see above, point 2), representing the closest prior art, is that claim 1 is directed to the identification of GTPase inhibitors rather than activators. The technical effect of this difference is the pre-screening of anti-tumour drugs, considering that constitutively active forms of GTPases locked in the GTP-bound state are involved in several human cancers (see description, page 2, para.2 and page 3, para.3). The objective technical problem of claim 1 in view of the closest prior art is therefore to identify GTPase inhibitors which are potential anti-cancer drugs. The solution proposed is a method comprising incubating a live cell expressing the GTPase and having a specific reporter thereof in presence of a test compound, and monitoring a change in the association between reporter and GTPase. This solution is considered to be not inventive. The skilled person would be aware of the high relevance of GTPase inhibition in relation to cancer treatment (see above), and would therefore be prompted to try and identify GTPase inhibitors as potential anti-cancer drugs. The skilled person would therefore tackle the technical problem of the application. As regards the solution to the technical problem, it would be obvious to the skilled person how to modify the disclosed methods (D1-D3) to adapt them to the identification of GTPase inhibitors (rather than activators): it suffices to detect a fluorescence decrease at the plasma membrane (where the GTPase is located) rather than a fluorescence increase in presence of the test compound (potential inhibitor).

4.1. Claims 3-5 and 50-54 are also considered not inventive for analogous reasons (see above, point 4). In particular, claims 4 and 5 are directed to methods for identifying compounds inhibiting GTP loading of GTPases, but the methods comprise exactly the same steps as the method of claim 3 for identifying GTPase

inhibitors, irrespective of the specific GTPase function inhibited. The method of claims 4 and 5, as well as the method of claim 3, only detects a change in the association of a GTPase with a reporter molecule induced by a test compound, and therefore does not appear to be suitable for identifying inhibitors which act with a specific mechanism of action (inhibition of GTP loading), but only to identify inhibitors in general, irrespective of the mechanism. As the methods of claims 4 and 5 do not appear to solve the problem (identification of inhibitors of GTP loading), an inventive step cannot be acknowledged for claims 4 and 5.

5. The subject-matter of claim 55, with the limitation discussed above in Item III, is not novel (Art.54 EPC). D4 (see figure 1b) discloses the amino-acid sequence of the protein CAPRI, comprising the sequences of some of the peptides listed by pages 48-49 of the description, e.g. KDRNGTSDPFVRV. However, peptides shorter than or equal to 20 amino acids (as discussed in the description, page 31, line 29-page 32, line 5) and comprising the sequences of pages 48-49 of the description are novel, as they are not disclosed by the cited prior art. An inventive step can however be acknowledged only for the peptides for which a function has been demonstrated, i.e. the peptides of pages 48-49 (activation or inhibition of small membrane-bound GTPases). For no other peptide does the application provide disclosure of a function, and therefore an inventive step cannot be acknowledged.

As regards claims 56-59, related to the medical uses of such peptides, the application provides no evidence of a medical use whatsoever in relation to the peptides identified. The fact that said peptides activate or inhibit small membrane-bound GTPases does not imply a therapeutic efficacy of the peptides themselves. Therefore an inventive step cannot be acknowledged.